Extracts of *Ginkgo biloba* or *Artemisia* species reduce feeding by neonates of codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), on apple in a laboratory bioassay

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ABSTRACT

In a simple bioassay, alcohol extracts (91% v/v ethanol, 4% v/v methanol, 5% v/v isopropanol) from *Ginkgo biloba* L., *Artemisia absinthium* L., *A. arborescens* L. x *A. absinthium* L., and *A. ludoviciana* Nutt. "Valerie Finnis", significantly reduced feeding of neonatal codling moth larvae on apple. Extracts from *A. californica* Less. and *A. vulgaris* L. had no effects.

Key Words: insect feeding, larvae, repellent, deterrent

INTRODUCTION

Codling moth, *Cydia pomonella* (L.), is a cosmopolitan pest that primarily attacks apple, *Malus domestica* Borkh., a commodity worth more than US$1 billion per year in the northwestern United States and British Columbia, Canada. Codling moth females oviposit mostly on foliage (Jackson 1979). Newly hatched neonates travel over apple branches and foliage in search of the fruit (Jackson 1982) and finally burrow into it (Tadic 1957). Manipulation of neonate searching behaviour may provide an alternative or complementary approach to current strategies for codling moth management (Pszczolkowski 2007).

Neonate codling moth responses to feeding stimulants have previously been explored (Pszczolkowski 2007). Suomi *et al.* (1986) and Landolt *et al.* (1999) studied deterrent effects of, respectively, plant extracts and plant essential oils. Of the 25 species tested by Suomi *et al.* (1986), five showed promise as feeding deterrents to neonatal codling moth larvae: absinthe wormwood, *Artemisia absynthium* L.; rabbitbrush, *Chrysothamnus nauseosus* (Pall. ex Pursh) Britton; false hellebore, *Veratrum californicum* Durand; garlic, *Alium sativum* L.; and tansy, *Tanacetum vulgare* L. Of the 27 species tested by Landolt *et al.* (1999), the greatest arrestment of neonates was achieved with oils of lavender, *Lavandula officinalis* Chaix ex Vill; pennyroyal, *Mentha pulegium* L.; and cypress, *Cupressus sempervirens* L. Oils of rue, *Ruta graveolens* L.; garlic; patchouly, *Pogostemon cablin* (Blanco) Benth.; and tansy were the most repellent to neonates (Landolt *et al.* 1999). Extracts from the ginkgo tree, *Ginkgo biloba* L., which have deleterious effects on codling moth neonates (Pszczolkowski and Brown 2005), were not studied by Suomi *et al.* (1986) or Landolt *et al.* (1999).

In this paper, we use a simple modification of the assay designed by Suomi *et al.* (1986) to test the effects of extracts of *G. biloba* and of five members of the genus *Artemisia* on feeding by codling moth neonates. Our assay allows small volumes of plant extracts on apple plugs to be presented to individual neonates. We compare

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neonates' response to gingko extract in the apple plug assay with their response to whole apples using an assay previously described in Pszczolkowski and Brown (2005).

MATERIALS AND METHODS

Insects. Codling moths originated from USDA-ARS Yakima Agricultural Research Laboratory in Wapato, WA, the same source as used by Suomi *et al.* (1986). This laboratory has maintained the codling moth colony for about 40 years, comprising more than 480 generations. Moths were held at 25 °C, 70-80% RH, under a 16L:8D light-dark regime, and allowed to oviposit on polypropylene foil. Neonates were collected 0.5-1 h post-hatch, and used for experiments.

Plant extracts. The extracts were prepared from foliage of *G. biloba*, *A. absinthium*, *Artemisia arborescens* L. x *Artemisia absinthium* L., *Artemisia ludoviciana* Nutt. "Valerie Finnis, *Artemisia californica* Less. and *Artemisia vulgaris* L.

Dried *A.absynthium* foliage was purchased from a local pharmacy (London Apothecary, Mansfield, MO). Remaining plant material was collected in the gardens of Missouri State University Research Campus, Mountain Grove, MO, in July 2008.

Dehydration alcohol (91% v/v ethanol, 4% v/v methanol, 5% v/v isopropanol; EMD Chemicals Inc., Gibbstown, NJ) was used to prepare all extracts. Plants were dehydrated using an Open Country food dehydrator (Nesco/American Harvest®, Two Rivers, WI) at 35 °C for 48 hours. The dry foliage was ground in a coffee grinder. Approximately 0.5 ml of dry plant powder was placed in a plastic centrifugation tube, 500 µl of dehydration alcohol added, then the tube was vortexed and left at room temperature for 10 min. The tube was then centrifuged at 2000 G for 10 min and 300 µl of liquid fraction was transferred to a pre-weighed plastic test tube and allowed to air dry overnight. The test tube with the residue was re-weighed the next morning and enough dehydration alcohol was added to make a 10 mg/ml solution of each plant extract. The extracts were prepared immediately before testing.

Modified assay using apple plugs. For each test arena, four plugs were procured from the same Golden Delicious apple, using a length of plastic soda straw (Fig. 1A), such that the straw covered the pulp, but not the epidermis of the apple. The crevice between the plug and the edge of the straw was sealed with paraffin wax applied with a warm spatula (Fig. 1B). The straws were then placed in a holder, apple plug facing up, and 5 µl of test solution were applied to each plug. The plugs were allowed to air dry, and four plugs were placed in a 60 x 15 mm polystyrene Petri dish (Fig. 1C). Small pieces of modeling clay held the plugs in place. New clay was used for each assay. A glass rod (1.3 mm diameter, 25-27 mm long) was positioned such that each end of the rod touched both the control and the treated member of the plug pair (Fig. 1C). One neonate was placed, using a camel-hair artistic brush, in the middle of the glass rod and the Petri dish was covered with a lid. The entire assembly was covered with a half of a white plastic RipBall (TM & Enor Corp., Northvale, NJ) to provide a white, slightly opaque cupola (Fig. 1D) and placed on the testing bench illuminated by fluorescent tubes and Soft White 60 general purpose bulbs (General Electric Canada, Cleveland, OH). Such an arrangement provided dispersed, non-directional light of uniform luminosity (900-920 lux) over each test arena, which was important because codling moth neonates exhibit mild phototropism (Jackson, 1982). Prior to every experiment, glass rods and Petri dishes were washed sequentially in tap water, double distilled water, alcohol, then dried.

Preliminary experiments showed that neonates could be expected to locate a plug and begin feeding within 20 hours, and that

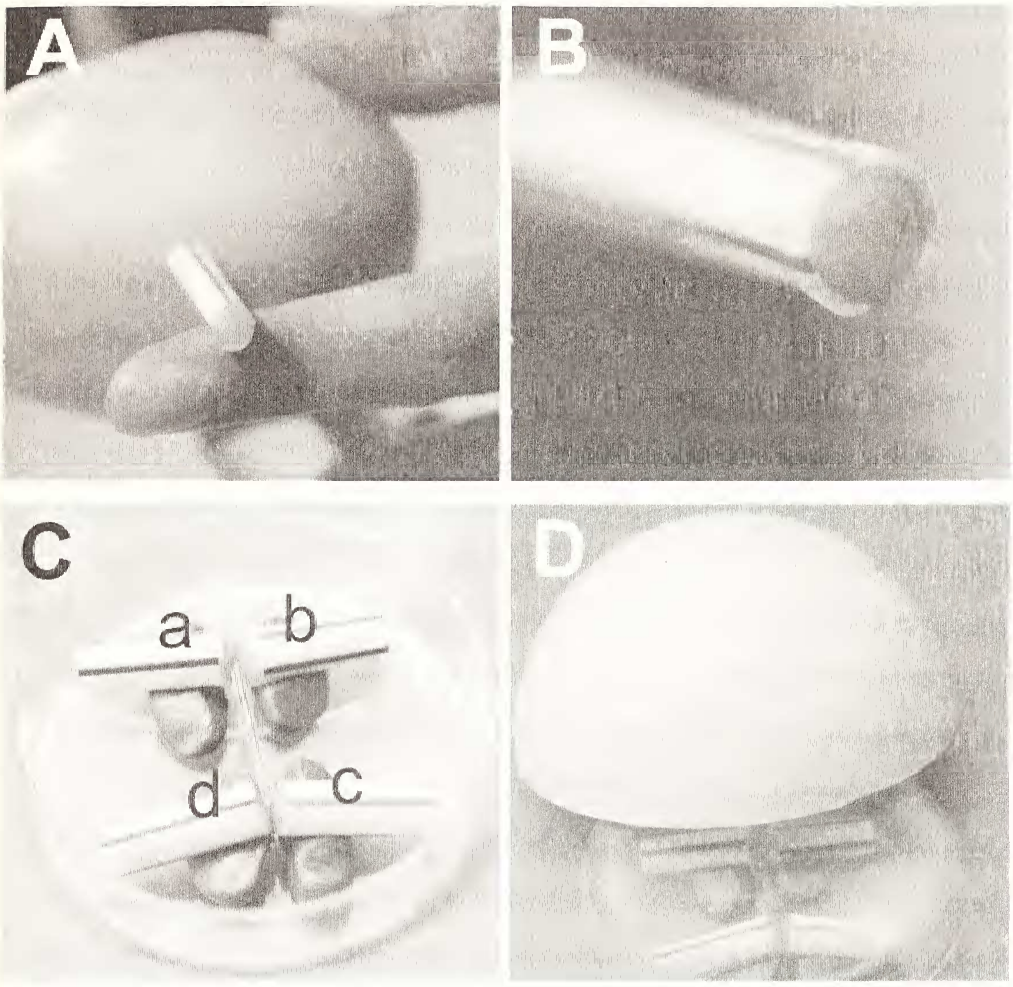

Figure 1. Preparation of test arena (60 mm diameter) for apple-plug assay. A. Cutting the plug out of apple. B. Crevice between the plug and the straw is sealed with paraffin wax. C. a, c. Plugs treated with plant extracts; b, d. control plugs treated with dehydration alcohol. D. Test arena is covered with plastic cupola to disperse light and eliminate possible effects of directional lighting.

neonates showed no preference for any plug position in the arena. In the experiments reported here, 30 neonates were individually exposed to ginkgo extracts, and 18-23 neonates were individually exposed to extracts of each *Artemisia* species. All neonates were tested simultaneously. All apple plugs were examined under a dissecting microscope after 20 h for feeding indicators such as abraded epidermis, presence of excrement, or a feeding cavity. If evidence of feeding was found, the plug was removed from the straw and dissected to reveal the larva.

Whole-fruit assay. To test whether or not neonates behaved similarly when presented with ginkgo extracts on whole fruit, we compared neonate behaviour in the apple-plug assay with behaviour in our previously described whole-fruit assay (Pszczolkowski and Brown, 2005). Uninfested thinning apples (Red Delicious; about 20 mm diameter) from Mountain Grove experiment orchards were used for whole-fruit assays. Apples were submerged in 10 mg/ml extract of ginkgo in dehydration alcohol or in dehydration alcohol only (about 200 µl of test solution per apple),

and allowed to air dry. Two apples (one ginkgo-treated and one alcohol-treated control) were placed 0.5 cm apart in a 70-mm diameter Pyrex glass crystallizing dish. One neonate was gently placed with a fine camel-hair artistic brush in the space between the fruits and the crystallizing dish was covered with a glass Petri dish. To prevent airflow that could bias the results of the assay, the entire assembly was placed in a semi-translucent 473-ml high-density polypropylene container and covered with a transparent lid. The testing bench and test arenas were illuminated as described for the apple-plug assay. Before each test, glassware and polypropylene containers were washed sequentially in tap water, double distilled water, alcohol, then dried.

Thirty neonates were tested individually in this assay. Both apple-plug assay and whole-fruit assay were conducted at the same time After 24 h, all apples were examined under a dissecting microscope for evidence of feeding, as described above for the apple-plug assay.

Statistical analysis. Exact Fisher's test (α=0.05) was used in all assays to test the null hypothesis that neonates do not discriminate between plugs or apples treated with plant extract and those treated with alcohol (i.e., 50% of the neonates choose treated plugs or apples and 50% of the neonates choose control plugs or apples).

RESULTS

Effects of ginkgo in apple-plug assay and whole-fruit assay. In both the apple-plug and the whole-fruit assays, the majority (29 of 30, and 28 of 30, respectively) of neonates avoided fruit treated with 10 mg/ml of ginkgo extract ($P< 0.001$). For every neonate, feeding indicators such as abraded epidermis, presence of excrement, or feeding cavities were found on only one apple plug or one apple out of two members of one pair. We conclude that this is evidence that each neonate larva, upon arrival at a ginkgo-treated plug or fruit, did not attempt to feed and was deterred or repelled, or both, by ginkgo extract.

Effects of Artemisia extracts in apple-plug assay. Extracts from three of the five *Artemisia* species discouraged neonates from burrowing into apple plugs (Table 1). Extracts from *A. absinthium, A. arborescens x A. absinthium* and *A. ludoviciana* "Valerie Finnis" were active ($P<0.01$). *Artemisia vulgaris* and *A. californica* had no effect. As in the case of ginkgo, for every neonate, feeding indicators were found on only one apple plug out of two members of one pair. We conclude that *Artemisia* extracts were either repellent or deterrent or both.

DISCUSSION

Deterrent or repellent activity of ginkgo extracts toward codling moth neonates is a novel finding. Surprisingly, information about insect deterrent activity of this plant is scarce in the literature, but what exists provides indirect supportive evidence. Two studies showed that extracts from ginkgo foliage reduce feeding by two insect pests of cabbage: *Pieris brassicae* (Fu-Shun *et al.* 1990) and *P. rapae* (Matsumoto and Sei 1987). Addition of anacardic acids (an alkylphenol found in ginkgo) reduced intake of artificial diet in Colorado potato beetle, *Leptinotarsa decemlineata* (Schultz *et al.* 2006). Other biologically active components of ginkgo foliage include flavonoids and there is evidence that some flavonoids have deterrent and antifeedant activity in insects (Simmonds 2001). At the current stage of our study, we do not know what constituents of ginkgo extract discourage codling moth larvae from burrowing into apple plugs.

The finding that *A. absinthium* deters codling moth neonates corroborates the results of Suomi *et al.* (1986). In their experiments, only 9% of larvae bored into apple plugs treated with a 1% extract ob-

Table 1.
Effect of 10 mg/ml extracts obtained from plants in the genus *Arthemisia* on feeding by codling moth neonates.

Plant species used for apple plug treatment	Number of larvae tested	Number of larvae feeding on treated apple plugs[1,2]
Artemisia absinthium	21	2 **
Artemisia arborescens x A. absinthium	16	1 **
Artemisia ludoviciana "Valerie Finnis"	17	1 **
Artemisia vulgaris	16	4 †
Artemisia californica	18	7 †

[1] ** $P<0.01$, Fisher's exact test
[2] † no statistical significance

tained from this plant. Our results showing that extracts from other members of *Artemisia* genus also have deterrent properties against codling moth neonates are novel, but not surprising in the light of other data from the literature. For instance, the essential oil of *A. annua* has repellent activities against two economically important stored-product pests, the red flour beetle *Tribolium castaneum* (Herbst) and the cowpea weevil *Callosobruchus maculatus* (Tripathi *et al.* 2000). The compound 1,8-cineole isolated from the same plant has feeding deterrent activity against *T. castaneum* (Tripathi *et al.* 2001). Essential oils from *A. vulgaris* also repel *T. castaneum* beetles (Wang *et al.* 2006). The fact that different plants from the same genus have different biological activity against codling moth neonates may facilitate isolation of their active components by comparative chemical analysis.

We think that our findings warrant further studies on effects of ginkgo and *Artemisia* extracts on codling moth neonates. Active constituents of these extracts should be identified, and their potential as codling moth feeding deterrents or repellents – assessed. Perhaps, if manufactured on a larger scale, those constituents could be used as organic alternatives to conventional insecticides for management of codling moth on apples.

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REFERENCES

Fu-Shun, Y., K.A. Evans, L.H. Stevens, T.A. van Beek and L.M. Schoonhoven. 1990. Deterrents extracted from the leaves of *Ginkgo biloba*: effects on feeding and contact chemoreceptors. Entomologia Experimentalis et Applicata 54: 57-64.

Jackson, M.D. 1979. Codling moth egg distribution on unmanaged apple trees. Annals of the Entomological Society of America 72: 361-368.

Jackson, M.D. 1982. Searching behavior and survival of 1st-instar codling moths. Annals of the Entomological Society of America 75: 284-289.

Landolt, P.J., R.W. Hofstetter, and L.L. Biddick. 1999. Plant essential oils as arrestants and repellents for neonate larvae of the codling moth (Lepidoptera: Tortricidae). Environmental Entomology 28: 954-960.

Matsumoto, T. and T. Sei. 1987. Antifeedant activities of *Ginkgo biloba* L. components against the larva of *Pieris rapae crucivora.* Agricultural and Biological Chemistry 51: 249-250.

Pszczolkowski, M.A. 2007. How to trick codling moth? Prospects of manipulation with neonate larvae

behavior to the benefit of the grower. Annals of Warsaw University of Life Sc. – SGGW, Horticulture And Landscape Architecture 28: 7-18.

Pszczolkowski M.A. and J.J. Brown. 2005. Single experience learning in host fruit selection by lepidopteran larvae. Physiology and Behavior 86: 168-175.

Schultz D.J., C. Olsen, G.A. Cobbs, N.J. Stolowich, and M.M. Parrott. 2006. Bioactivity of anacardic acid against colorado potato beetle (*Leptinotarsa decemlineata*) larvae. Journal of Agricultural and Food Chemistry 54: 7522-7529.

Simmonds, M.S.J. 2001. Importance of flavonoids in insect-plant interactions: feeding and oviposition. Phytochemistry 56: 245-252.

Suomi, D., J.J. Brown and R.D. Akre. 1986. Responses to plant extracts of neonatal codling moth larvae, *Cydia pomonella* (L.), (Lepidoptera: Tortricidae: Olethreutinae). Journal of Entomological Society of British Columbia 83: 12-18.

Tadic, M. 1957. Jabucni Smotavac (*Carpocapsa pomonella* L.). Biologija kao osnova za njegovo suzbijanije. Univerzitet u Beogradu, Belgrade, Yugoslavia. 100 pp.

Tripathi, A.K., V. Prajapati, K.K. Aggarwal, S.P.S. Khanuja and S. Kumar. 2000. Repellency and toxicity of oil from *Artemisia annua* to certain stored-product beetles. Journal of Economic Entomology 93: 43-47.

Tripathi, A.K., V. Prajapati, K.K. Aggarwal and S. Kumar. 2001. Toxicity, feeding deterrence, and effect of activity of 1,8-cineole from *Artemisia annua* on progeny production of *Tribolium castaneum* (Coleoptera: Tenebrionidae). Journal of Economic Entomology 94: 979-983.

Wang, J., F. Zhu, X.M. Zhou, C.Y. Niu and C.L. Lei. 2006. Repellent and fumigant activity of essential oil from *Artemisia vulgaris* to *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Journal of Stored Product Research 42: 339-347.